Effects of a novel method of acute tryptophan depletion on plasma tryptophan and cognitive performance in healthy volunteers.

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ORIGINAL INVESTIGATION

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Abstract *Rationale:* Disorders associated with low levels of serotonin (5-HT) are characterized by mood and cognitive disturbances. Acute tryptophan depletion (ATD) is an established method for lowering 5-HT levels and an important tool to study the effects of reduced 5-HT on mood and cognition in human subjects. The traditional ATD method, i.e., administration of separate amino acids (AAs), has several disadvantages. The AA mixture is costly, unpalatable and associated with gastrointestinal discomfort. *Objectives:* The University of Maastricht developed a new and inexpensive method for ATD: a natural collagen protein (CP) mixture with low tryptophan (TRP) content. The reductions in plasma TRP after taking this CP mixture were compared with the reductions achieved taking the traditional AA mixture, and effects on memory and reversal learning were studied. *Methods:* Fifteen healthy young volunteers participated in a double-blind, counterbalanced within-subject study. Reversal learning, verbal memory and pattern recognition were assessed at baseline and 3–4 h after taking the CP mixture. *Results:* The new ATD method significantly reduced plasma TRP by 74% and the ratio between TRP and the other large AAs (TRP/LNAA) by 82%. The placebo mixture did not change these measures. Delayed recognition reaction time on the verbal learning task was increased following ATD. No other cognitive effects were found. *Conclusions:* The CP mixture was shown to be an efficient tool for lowering plasma TRP in humans. The validity of this method with regard to behavioral changes remains to be established in healthy, vulnerable and clinical populations.

Keywords Serotonin · Acute tryptophan depletion · Memory · Reversal learning · Mood

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Introduction

Serotonergic (5-HT) dysfunction is associated with disrupted cognitive function, emotional processing and social functioning, which are characteristic for depressive patients (Murphy et al. 2002). A pharmacological model to study the role of 5-HT in human behavior is acute tryptophan depletion (ATD), in which central 5-HT synthesis is reduced lowering the brain availability of the 5-HT precursor L-tryptophan (TRP) (reviewed by Reilly et al. 1997). Animal (Biggio et al. 1974; Gartside et al. 1992) and human (Nishizawa et al. 1997, Carpenter et al. 1998; Williams et al. 1999) studies have demonstrated that the acute reduction of TRP to the brain is sufficient to produce a rapid decrease in the synthesis and release of brain 5-HT.

Previous ATD studies showed impaired performance on tasks involving memory consolidation (see Riedel et al. 2002 for an overview), decision making (Rogers et al. 1999, 2003), reversal learning (Murphy et al. 2002; Rogers et al. 1999) and affective processing (Murphy et al. 2002; Rubinzstein et al. 2001) in healthy volunteers. The aim of the present study was to investigate the effects of a novel ATD method on the plasma TRP levels, on the ratio between TRP and the other large neutral amino acids (ΣLNAAs), and to validate the method on a behavioral level. To this end, we assessed several cognitive functions that are known to be sensitive to ATD, i.e., memory and reversal learning.

A low-TRP collagen-protein (CP) mixture was used to induce an ATD. This protein is derived from the selective hydrolysis of CP and comprises the entire range of amino acids (AAs) in the form of peptides. After administration, these peptides are decomposed into AAs, and the mechanism of depletion is identical to that of the AA mixture. In rats, the CP mixture significantly lowered plasma TRP and TRP/LNAA ratios (−78%) and brain TRP and 5-HT (−50%) concentrations (Lieben et al. 2004). Based on the previous behavioral ATD studies and the demonstration that the CP mixture was an efficient TRP-depletion method in rats, it was hypothesized that this new method of ATD results in reduced plasma TRP, a decreased TRP/LNAA ratio, impaired delayed recall and recognition in word and pattern-learning tasks, slower responding and more errors in the reversal-learning task when compared with the placebo in healthy volunteers.

Materials and methods

Subjects

Fifteen young healthy male (n=3) and female (n=12) volunteers (mean age 21.8 years; SD=1.8) gave informed consent and participated in this study, which was approved by the medical ethics committee of the University Hospital, Maastricht. The participants were free from significant past or present physical or psychiatric illness and did not use medication other than oral anti-conceptives. Female participants were not tested in the late luteal phase of the menstrual cycle (days 21–28). All subjects completed the study.

CP mixture

The CP mixture was purchased from PB Gelatins (Tessenderlo, Belgium); see Table 1 for the AA composition. To obtain a drinkable mixture, 100 g of the CP mixture was mixed with 200 g water. The placebo mixture was identical in composition, but 1.2 g L-TRP (Sigma, Zwijndrecht, The Netherlands) was added.

Table 1 Composition (grams) of the natural collagen protein (tryptophan$^-$) in 100 ml tap water

Aspartic acid + asparagines	5.2	Tyrosine	0.4
Glutamic acid + glutamine	9.3	Valine	2.1
Hydroxyproline	12.1	Methionine	0.6
Serine	3.1	Cysteine	0.2
Glycine	22.5	Isoleucine	1.4
Histidine	0.5	Leucine	3
Arginine	8.8	Hydroxylysine	1.4
Threonine	1.1	Phenylalanine	1.9
Alanine	9.3	Tryptophan	0.1
Proline	13.3	Lysine	3.6

Design

This study was conducted according to a double-blind, placebo-controlled crossover design. The participants received a TRP-free CP mixture (TRP$^-$) and a CP mixture with 1.21 g TRP added (placebo) on separate occasions. Treatment order was balanced over the two test days, which were separated by at least 3 days.

Procedure

In a separate session, approximately 1 week before the actual test days, the cognitive tasks were practiced to minimize learning effects. The participants were instructed not to drink alcohol on the days prior to the test days, not to eat or drink (except water) after 2200 hours that evening and to arrive at the laboratory well rested. A test day started with a cognitive test battery, subjective assessments of mood and adverse effects, and baseline blood sampling. Subsequently, subjects received the TRP$^-$ or the placebo mixture. A 3-h break followed to maximize TRP depletion. The participants had free access to low-TRP food, such as apples, tomatoes and protein-free candy, and caffeine-free tea during the pause. These food items were generally consumed in small quantities, and the intake of carbohydrates does not meaningfully affect brain TRP availability in the presence of large amounts of protein (Teff et al. 1989). After the 3-h interval, cognition, mood and adverse effects were assessed again and a blood sample was taken. The duration of a test day was 5 h in total.

Cognitive assessment

The cognitive test battery, mood and adverse effects assessments took approximately 1 h to complete. On each session parallel test versions were used, version order was distributed among the participants using a 4×4 Latin square.

Probabilistic reversal-learning task

In the reversal-learning task (described in detail by O' Doherty et al. 2001), two abstract stimuli, composed of two bars of different color, were randomly presented to the left or right side of a computer screen. One stimuli was advantageous (S+) and was usually (70%) associated with large reward (addition of 80–250 points) and occasionally (30%) with a small punishment (subtraction of 10–60 points), based on a pseudo-random sequence. The other stimulus was disadvantageous (S−) and usually (60%) associated with a large punishment (250–600 points) and occasionally (40%) a small reward (30–60 points). Volunteers had to determine which stimulus was advantageous based on the feedback—the number of points won or lost. Once this was learned, i.e., the advantageous stimulus was

chosen four times out of five responses, the stimulus-reward contingencies were reversed (S+ became S−, and S− became S+). The participant's task was to keep track of the most profitable stimulus and to collect as many points as possible. Two successive 8-min blocks were performed per session. The stimuli used in the two reversal blocks were four differently colored abstract patterns, and blocks were randomly assigned. The task stopped after the 19th reversal or after 121 trials. Before each assessment, subjects practiced an acquisition (eight correct responses in a row) and a short reversal-learning task (two reversals within 50 trials: 8 correct out of 9 trials). Dependent variables were the total number of reversals, the total number of preservations (number of errors directly after the rule has changed), the mean reaction time per trial, the mean reaction time of the first response following a reversal switch and the total number of points collected, in the two successive reversal blocks.

The visual verbal learning test

The visual verbal learning test (VVLT), a modified version of the Rey Auditory Verbal Learning Test (Lezak 1995), consisted of a list of 30 monosyllabic words in Dutch, which were presented in three trials on a computer screen (Riedel et al. 1999). Of the words, 12 were positively loaded, 12 were negatively loaded and 6 were neutrally loaded. Immediately after each presentation (immediate recall) and 30 min later (delayed recall), the subjects were asked what words they could remember. After that, a delayed recognition test was presented; 30 words were shown (15 new and 15 words from the original word list), and the subject was asked to press "yes" for "familiar" and "no" for "new", as quickly as possible. According to the theory of signal detection (Pollack and Norman 1964), the proportion of correctly recognized words (cr) and the proportion of those falsely recognized (fr) constituted the non-parametric sensitivity measure: $A' = 1 - 1/4(\mathrm{fr}/\mathrm{cr} + (1 - \mathrm{cr})/(1 - \mathrm{fr}))$ where A is in fact the proportion of correctly recognized words corrected for the participant's response tendency. A was arc sin transformed before statistical analyses.

The outcome variables were the number of correct words recalled during the three immediate recall trials as a measure of short-term memory, the number of correct words produced on delayed free recall as a measure of retrieval from long-term memory, A' as a measure of storage in long-term memory, and the medial reaction times of correctly recognized words as a measure of speed of long-term memory.

Abstract pattern-learning task

In the abstract pattern-learning task, 15 abstract stimuli were shown one by one on a computer screen, followed by the recognition task in which the participants had to press the left button of a response box if they recognized the left stimulus and the right button if they recognized the right stimulus. Recognition was tested immediately after the presentation of the patterns (immediate recognition) and 30 min later (delayed recognition). The dependent measure of interest was the proportion correctly recognized patterns and the reaction times of the correct responses.

Questionnaires

Mood

A visual analogue version of the profile of mood states (POMS) was used to assess mood (McNair et al. 1988). This questionnaire consists of 32 bipolar sets of adjectives, which measure five mood dimensions: anger, depression, fatigue, tension and vigor. The items were scored on a 0- to 100-mm scale. When the participants felt as they

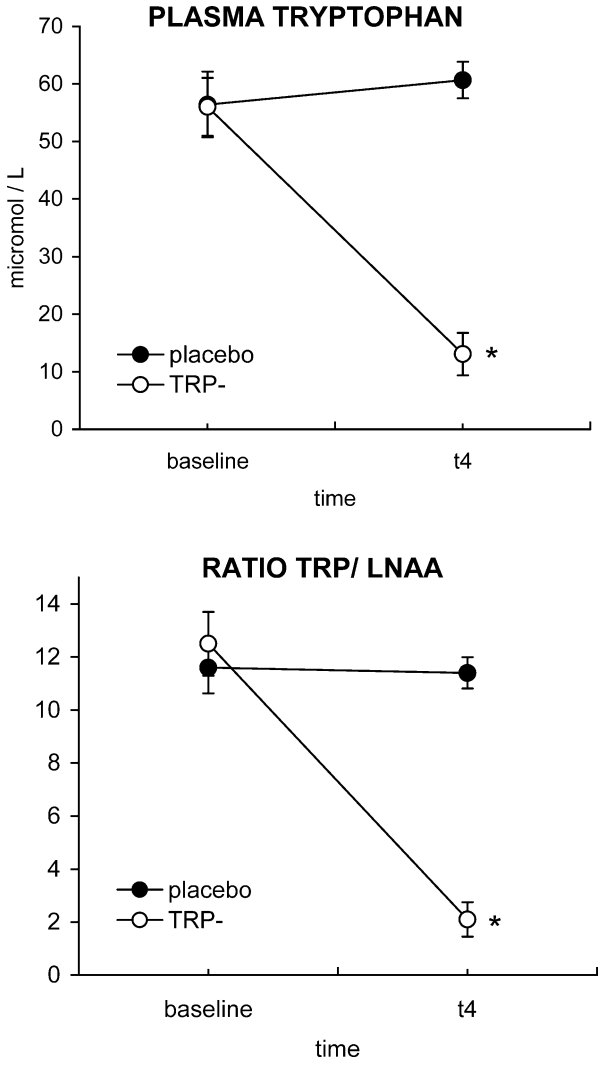

Fig. 1 The means and the standard errors (SE) of the blood plasma levels of tryptophan (μmol/l) and the ratio between tryptophan and the other large amino acids (TRP/LNAA) at baseline and 4 h after start of the TRP depletion and placebo treatment. *P<0.001

Table 2 Mean scores (standard deviations) for the outcome variables of the visual verbal learning task, the probabilistic reversal-learning task and the abstract visual learning task at baseline and 3–4 h after administration of the tryptophan (TRP)$^-$ and placebo mixture

	TRP$^-$ condition		Placebo condition	
	Baseline	T3–4	Baseline	T3–4
Visual verbal learning task				
Number of words correctly recalled on the three immediate recall trials				
Trial 1	12.07 (3.8)	9.4 (3.3)	11.9 (3.1)	10.3 (2.7)
Trial 2	17.5 (4.4)	15.0 (4.9)	18.1 (3.6)	16.7 (3.8)
Trial 3	21.5 (3.9)	18.9 (7.7)	21.7 (3.7)	20.4 (4.2)
Number of words correctly recalled on delayed recall	18.7 (6.2)	13.7 (7.0)	19.7 (6.0)	15.0 (5.5)
Delayed recognition sensitivity measure A (percentage)	96 (4)	94 (6)	97 (2)	95 (6)
Median reaction time of correctly recognized words (ms)	677 (92)	**703** (98)	669 (69)	**654** (74)
Probabilistic reversal-learning task				
Total number of reversals	18.0 (1.5)	18.3 (1.3)	18.1 (1.1)	18.6 (1.1)
Total number of preservations	26.0 (7.9)	25.1 (6.9)	25.5 (6.6)	23.1 (6.7)
Mean RT	496 (88)	479 (109)	464 (89)	426 (64)
Mean RT after a reversal	487 (90)	474 (114)	461 (94)	436 (76)
Total number of points	1381 (1217)	1451 (1674)	1400 (1776)	1732 (2173)
Abstract pattern recognition task				
Proportion correctly recognized patterns				
Immediate recognition	90.6 (11.0)	89.6 (14.0)	92.6 (6.2)	93.1 (8.1)
Delayed recognition	91.0 (8.3)	82.8 (15.6)	88.3 (8.2)	85.6 (11.7)
Reaction times for the correct responses				
Immediate recognition	1604 (451)	1613 (498)	1580 (434)	1593 (436)
Delayed recognition	1593 (411)	1758 (486)	1503 (372)	1597 (530)

normally do, they were asked to mark the middle of the line (score 50).

Adverse effects

Adverse effects, 31 items, were registered and scored on a five-point scale from "no complaint at all" (0) to "severe complaint" (4).

Plasma AAs

In total, four blood samples (4 ml) were drawn from each volunteer and immediately centrifuged at 4°C (10 min, 4500 rpm). A 100-µl aliquot of plasma was mixed with 8 mg sulfasalicyl acid and frozen at −80°C until AA analysis using high-performance liquid chromatography (van Eijk 1993).

Statistical analysis

The outcome variables of the cognitive and mood assessments, and the total number of adverse effects were analyzed using a repeated-measures analysis of variance (ANOVA) of the difference scores (performance scores 3–4 h after the drink administration minus baseline scores) using treatment (TRP$^-$, placebo) as within-subject factor and treatment order (TRP$^-$ or placebo mixture first) as between-subject factors.

Results

Levels of plasma TRP

Blood samples of two participants were missing. Figure 1 shows that plasma TRP concentrations and TRP/ΣLNAA ratios at baseline did not differ between groups. Three hours after drinking the TRP$^-$ mixture, plasma TRP was significantly reduced by 74% ($F_{1,11}$=60.8, P<0.001) and the TRP/ΣLNAA ratios by 82% ($F_{1,11}$=50.4, P<0.001), compared with baseline. Small, non-significant changes in

Table 3 Mean (standard deviation) scores on the subscales of the profile of mood questionnaire at baseline and 4 h after administration of the tryptophan (TRP)-depleted mixture (TRP$^-$) and the placebo mixture

	TRP$^-$ condition		Placebo	
	Baseline	T4	Baseline	T4
Depression	5.6 (0.9)	5.5 (1.0)	5.5 (1.3)	5.4 (1.3)
Anger	5.7 (1.0)	5.6 (1.2)	5.6 (1.4)	5.5 (1.4)
Fatigue	5.1 (0.9)	4.9 (1.0)	4.9 (1.2)	4.8 (1.1)
Vigor	4.7 (0.8)	5.3 (1.0)	6.0 (3.4)	5.2 (0.9)
Tension	5.4 (1.0)	5.4 (0.8)	5.6 (1.4)	5.6 (1.3)

Table 4 The number of participants experiencing the physical complaint mentioned

Adverse effects	TRP^- condition		Placebo	
	Baseline	T4	Baseline	T4
Headache	3	2	4	6
Sleepiness	8	9	10	7
Dizziness	0	0	3	3
Nausea	1	4	1	4
Restlessness	2	3	5	3
Heart palpitations	0	0	0	0
Stomach ache	1	1	1	2
Bloating	2	5	0	5
Heartburn	0	2	0	1
Loss of appetite	0	5	1	5
Hunger	12	5	13	6
Diarrhea	0	1	0	0
Feeling cold	7	5	7	5
Feeling warm	1	1	0	0
Dry mouth	6	2	7	6
Trembling	0	0	2	2
Feeling tired	10	10	9	10
A hazy view	1	0	2	2
Sweating	0	0	0	1
Sedation	7	8	7	8
Feeling feeble	5	8	4	4
Tightness in the chest	1	0	0	0
Decreased concentration	3	8	3	11
Tingling	0	0	0	1
Nervousness	2	0	3	0
Irritation	1	1	0	0
Listless	3	2	1	4
Bothered by bright light	0	0	0	0
Bothered by hard sounds	1	0	0	0
A warm head	1	2	1	2
The feeling that you could faint	1	1	0	0

plasma TRP (+8%) and TRP/ΣLNAA ratio (−2%) were observed following administration of the placebo mixture.

Cognitive assessments

The results of the cognitive assessments are summarized in Tables 3, 4. Delayed recognition reaction time of the VVLT was increased following ATD ($F_{1,13}$=9.2, P=0.01). No treatment effects were seen on delayed recognition accuracy, delayed recall or overall immediate recall.

No effects of ATD were seen on the outcome variables of the probabilistic reversal-learning task and the abstract pattern-learning task. None of the analyses showed an interaction between treatment order and the effects of the treatment (Table 2).

Adverse effects and mood assessment

None of the subscales of the POMS—depression, anger, tension, fatigue and vigor—showed an effect of treatment (Table 3). As is shown in Table 4, neither the TRP^- nor the placebo mixture was associated with robust adverse effects at T4. None of the participants scored above "1" (bothered a bit) on any of the adverse effect items. The total adverse-effects score was identical in the ATD versus the placebo condition. Treatment order did not modify the outcome of the analyses.

Discussion

The TRP^- CP mixture appears to be an efficient tool to lower plasma TRP in humans. Four hours after administration, plasma TRP concentrations were reduced by 74% and the ratio TRP/ΣLNAA by 82%. These levels of TRP depletion are comparable to those obtained by administration of the "classic" AA mixture (Table 5). Furthermore, the present results show that our placebo mixture is es-

Table 5 The decrease in total plasma tryptophan (TRP), the ratio between TRP and the other large amino acids (ratio) in the tryptophan depletion (TRP^-) and the placebo conditions as a result of acute TRP depletion (ATD) by amino acid administration and the collagen protein (CP) method. A selection of recent studies using the traditional ATD method is shown in which the level of depletion was reported or could be calculated. For each study, the amount of amino acids (AA) and tryptophan (TRP) in grams (g), and the number of hours (t) between consuming the mixture and the providing blood samples are given. The last study mentioned is the present study in which a CP mixture was used

References	Plasma TRP		Ratio		AA (g)	TRP (g)	t
	TRP^- (%)	Placebo (%)	TRP^- (%)	Placebo (%)			
Riedel et al. (1999)	−67	+22	−78	−20	100	3.0	6
Schmitt et al. (2000)	−63	+80	−79	+20	100	4.6	5
Rubinsztein et al. (2001)	−80	+47			53	2.0	5
Murphy et al. (2002)	−70				86	1.9	7
Anderson et al. (2003)	−84	+118			100/86[a]	2.3/1.8[a]	6
CP-mixture	−74	+8	−82	−2	100	1.2	4

[a]100 g of AAs with 2.3 g TRP added for males and 86 g with 1.8 g TRP added for females.

sentially neutral with regard to effects on plasma TRP as well as the TRP/LNAA ratio. This constitutes an important advantage over placebo AA mixtures, in which neutral TRP/LNAA ratios can only be maintained at the cost of marked increases of TRP levels (van der Does 2001; Weltzin et al. 1994). Both TRP/LNAA (van der Does 2002; Moore et al. 2000) and free TRP levels (Biggio et al. 1974; Moja et al. 1989; Fadda 2000) have been suggested as being the predominant mediator of central TRP availability, meaning that changes in either parameter may result in an active control that may either underestimate or overestimate the effects of the depletion drinks (Reilly et al. 1997).

Although poorly documented, consumption of an ATD and placebo AA drink can be associated with quite unpleasant adverse effects, particularly gastrointestinal complaints with occasional vomiting, in some individuals. These adverse events occur predominantly in the first 1 h or 2 h after drink administration and subside in the ensuing hours, having little or no impact on assessments done 5 h or 6 h after the start of the treatment. However, these transient adverse effects can be quite discomforting for the subjects and may ultimately lead to subjects withdrawing from the study (Schmitt et al. 2000; Riedel et al. 1999; Danjou et al. 1990; Klaassen et al. 1999), while vomiting may diminish the level of depletion. It is clear that avoiding or minimizing adverse side effects would be highly desirable. In the present study, four subjects (all female) reported significant nausea, three of whom vomited, approximately 1 h after drink administration. Vomiting or nausea was not specifically related to either the TRP$^-$ (two occasions) or placebo (two occasions) mixtures. Vomiting did not substantially affect the level of TRP depletion: plasma TRP level was reduced by 80% and 94%, TRP/LNAA ratios by 95% and 95% in the two individuals mentioned. Further, excluding these subjects from analyses of the cognitive data did not affect the outcome. Although the current study was not specifically designed to investigate the time course, magnitude and prevalence of drink-related side effects—this would require multiple assessments over time and a non-drink group to ascertain time of day effects—it was speculated that the CP mixture would elicit minimal gastrointestinal effects because of its increased palatability and lack of separate AAs. Our current observations do not support this notion, but more systematic investigation is warranted. However, our data do clearly show that at the time of testing, i.e., 3–4 h after drink administration, the CP mixtures are not associated with significant adverse effects, which could interfere with performance and mood assessments.

Administration of TRP$^-$ CP protein induced a mild reduction of long-term memory function, which was apparent only in terms of reduced speed of delayed word recognition, but not accuracy. It is important to note that slowing of responses in the word recognition task cannot be attributed to a general reduction of psychomotor speed as no effects of ATD were seen on reaction time measures of other tasks. Overall, the memory effect is rather modest compared with previous findings showing impaired accuracy of delayed recall and/or recognition (Harrison et al. 2004; Sobczak et al. 2002; Riedel et al. 1999; Schmitt et al. 2000), as well as reduced speed of recognition (Riedel et al. 1999). Nevertheless, our findings are in line with accumulating evidence implicating 5-HT in long-term memory functioning (Riedel 2004; Buhot et al. 2000; Meneses 1999). It is therefore rather unexpected that long-term memory for abstract patterns was unaffected by ATD in the current study. Our results conflict with those reported by Rubinzstein et al. (2001), who found that ATD impaired delayed recognition of previously presented abstract patterns. However, in the latter study, feedback was given on the correctness of each response, and task performance may have been modulated by altered feedback processing following ATD. Elliott et al. (1996) showed that depressed patients [associated with reduced levels of serotonin (Maes and Meltzer 1995)] were oversensitive to negative feedback and in general showed a bias for negative stimuli (Murphy et al. 1999). The absence of ATD effects on this task, however, should also be viewed in the context of the overall picture of rather modest ATD effects, which may be related to more general methodological factors.

An overall explanation for the relatively mild long-term memory effects may be the timing of the post-treatment assessments. Generally, a 4-h to 5-h interval is maintained between ATD drink administration and subsequent testing, allowing for a maximal depletion of peripheral and presumably central TRP levels. A limited set of data from a series of pilot studies (unpublished data) suggested that maximal TRP depletion following TRP$^-$ CP administration was achieved after a 3-h to 4-h interval (Fig. 2). However, it is possible that, due to a delay between peripheral depletion and central 5-HT deficiency (Biggio et al. 1974), this time may be too short to produce robust

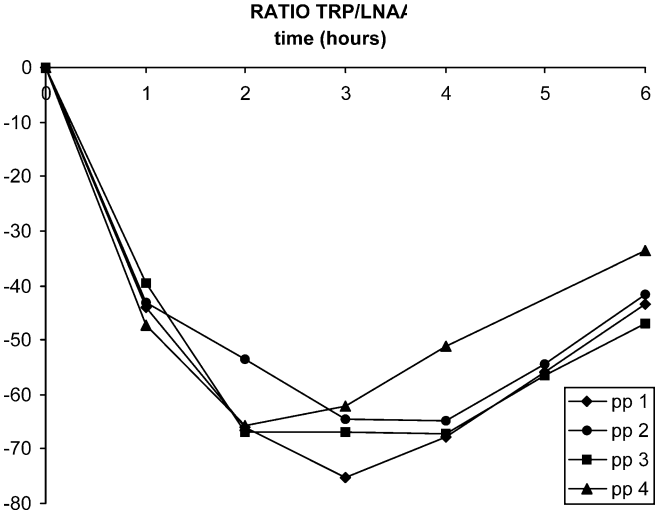

Fig. 2 The ratio between tryptophan and the other large amino acids (TRP/LNAA in percentage change) for four young healthy volunteers (p1–4) tested in a pilot experiment. Blood levels of TRP and the other large amino acids were measured at baseline and at hourly intervals until 6 h after consumption of the TRP$^-$ collagen protein mixture (100 g)

effects on memory function. In addition, it is possible that the use of an active control mixture that increases TRP levels leads to an overestimation of the cognitive effects of ATD in previous studies, since TRP depletion may have been compared with a state of enhanced TRP availability (Reilly et al. 1997). It can be argued that these factors may also underlie the absence of effect on the probabilistic reversal-learning task. However, other factors relating to the procedure and task characteristics may have also influenced the results. Task familiarity has been shown to modulate the effects of ATD on a number of tests of executive functioning (Gallagher et al. 2003), including probabilistic reversal learning (Murphy et al. 2002). It appears that ATD may diminish performance predominantly when the task is novel to the participants, e.g., on a single reversal switch on the first test day (Murphy et al. 2002). In this respect, it is important to note that the inclusion of separate practice sessions in the present study excludes any novelty effects. Furthermore, in the presented study, multiple reversal switches were assessed at each session as the reversal-learning task was designed to allow blocked fluorescence magnetic resonance imaging analyses in future research. It is always dangerous to draw conclusions based on negative results, especially when other factors may be involved, but the lack of ATD effects on reversal learning when novelty effects are excluded appears to be in line with the notion that the effects of ATD on probabilistic reversal learning may depend on task familiarity.

In conclusion, we have demonstrated that administration of a low-TRP collagen-based protein depletes peripheral TRP, decreases the peripheral TRP/LNAA ratio and presumably diminishes TRP availability in the brain. The biochemical effects are very similar to those observed after a traditional ATD that is based on AA drinks, but with a more neutral placebo with regard to both TRP and TRP/LNAA levels. Our data suggest that the CP method is a suitable alternative for the AA mixtures in TRP depletion research. Further studies will need to focus on the side-effect profile, optimal lag time for maximum biochemical and behavioral effects, and the validation of the method with regard to mood and cognitive changes in healthy, vulnerable and clinical populations.

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